

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
<small>Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.</small>				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 5/25/2001	3. REPORT TYPE AND DATES COVERED Final Report 01 Jul 98 - 30 Jun 00		
4. TITLE AND SUBTITLE Artificial Metallo-Phosphoesterase		5. FUNDING NUMBERS DAAG55-98-1-0398		
6. AUTHOR(S) Jik Chin				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Department of Chemistry, McGill University 801 Sherbrooke St. W. Montreal, Canada H3A 2K6		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Research Office PO Box 12211 RTP, NC 27709		10. SPONSORING/MONITORING AGENCY REPORT NUMBER 37670-CH ✓ 06		
11. SUPPLEMENTARY NOTES The view, opinions and/or finding contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation				
12a. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words) Our first two US Army contracts (July 1, '90 to June 30, '93 & Mar 15, '94 to Mar 14 '97) involved the study of mono and dinuclear metal complexes for hydrolyzing phosphate esters. We have now completed our third US Army contract. Due to my recent move from McGill University to University of Toronto (July 1, '00), the funding period for the third contract was reduced from three to two years (July 1, '98 to June 30, '00). During this period, we continued to study the role of metal ions in phosphate hydrolysis reactions with particular emphasis on understanding the hydrolysis of phosphates with good and poor leaving groups. We also studied the synergistic effect between metal coordination and H-bonding in phosphate hydrolysis. Finally, we have been able to dissect the role of metal ions in hydrolyzing phosphates. Generous funding from ARO for all of the above work is greatly appreciated!				
14. SUBJECT TERMS		15. NUMBER OF PAGES 6		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT unclassified	20. LIMITATION OF ABSTRACT UL	

20010608 075

MEMORANDUM OF TRANSMITTAL

U.S. Army Research Office
ATTN: AMSRL-RO-BI (TR)
P.O. Box 12211
Research Triangle Park, NC 27709-2211

Reprint (Orig + 2 copies)

Technical Report (Orig + 2 copies)

Manuscript (1 copy)

Final Progress Report (Orig + 2 copies)

Related Materials, Abstracts, Theses (1 copy)


CONTRACT/GRANT NUMBER: 37670-CH.

REPORT TITLE: Artificial Metallo-Phospho Esterases

is forwarded for your information.

SUBMITTED FOR PUBLICATION TO (applicable only if report is manuscript):

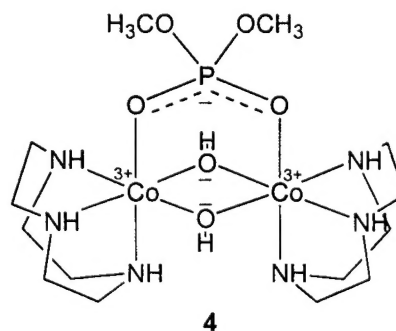
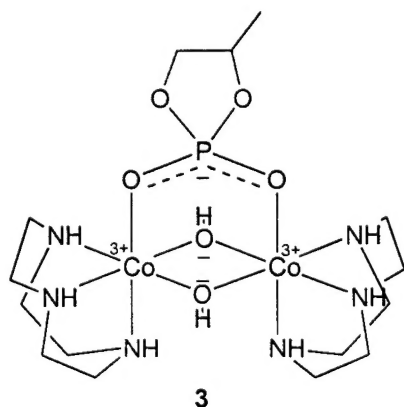
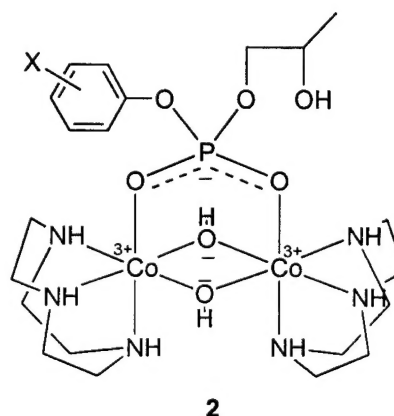
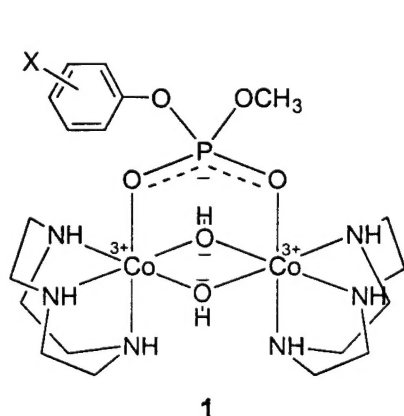
Sincerely,



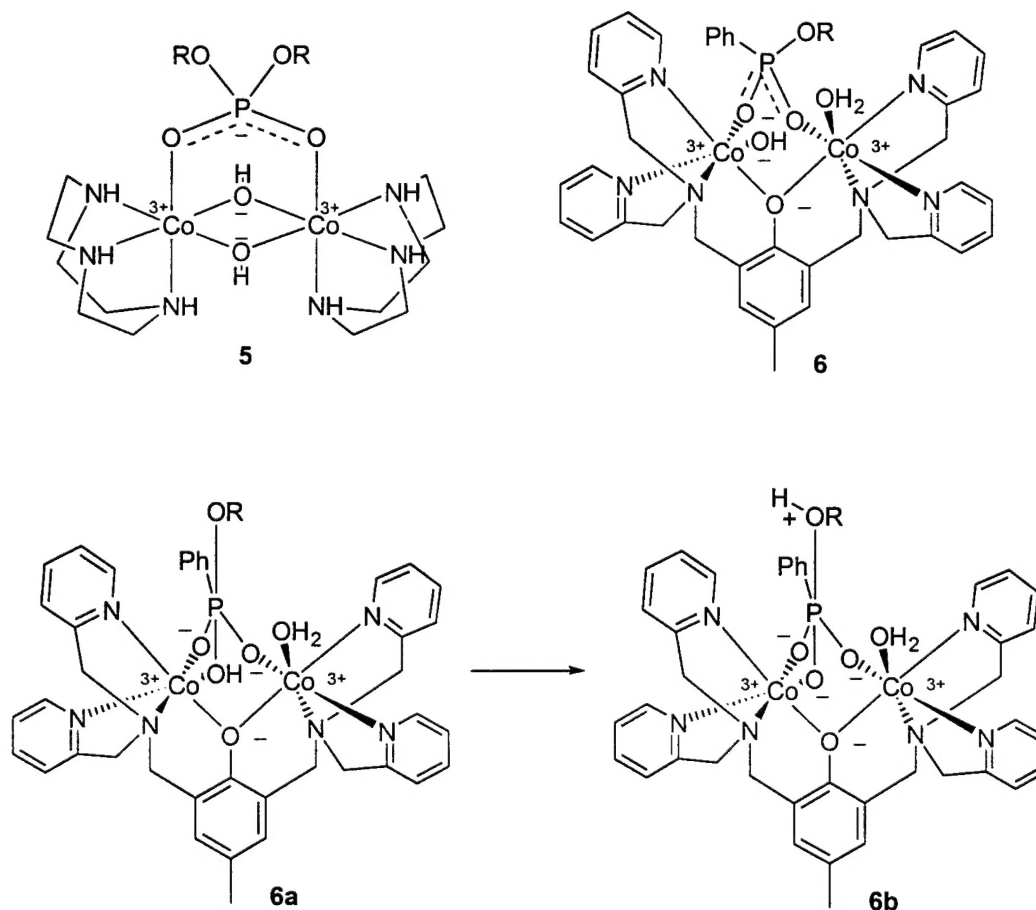
FINAL REPORT

Our first two US Army contracts (July 1, '90 to June 30, '93 & Mar 15, '94 to Mar 14 '97) involved the study of mono and dinuclear metal complexes for hydrolyzing phosphate esters. We have now completed our third US Army contract. Due to my recent move from McGill University to University of Toronto (July 1, '00), the funding period for the third contract was reduced from three to two years (July 1, '98 to June 30, '00). During this period, we continued to study the role of metal ions in phosphate hydrolysis reactions with particular emphasis on understanding *the hydrolysis of phosphates with good and poor leaving groups*. We also studied the synergistic effect between *metal coordination and H-bonding in phosphate hydrolysis*. Finally, we have been able to *dissect the role of metal ions in hydrolyzing phosphates*. Generous funding from ARO for all of the above work is greatly appreciated!

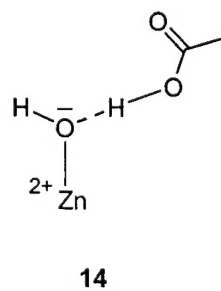
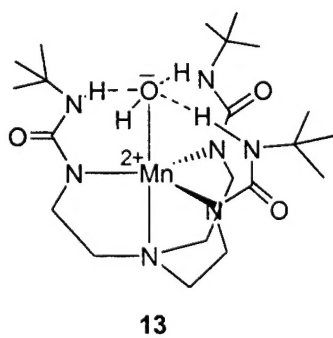
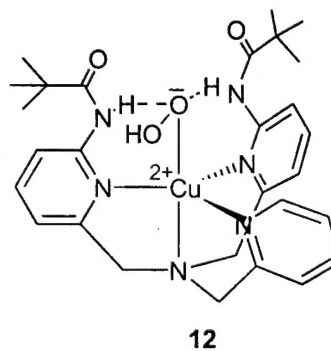
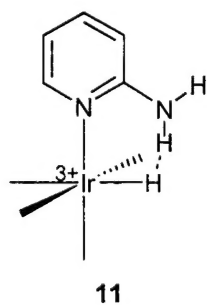
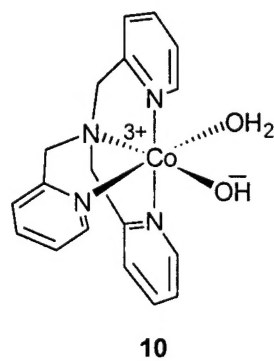
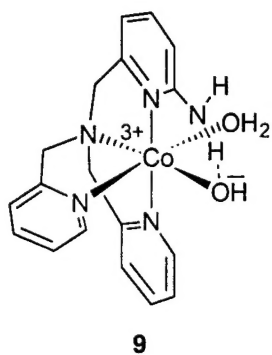
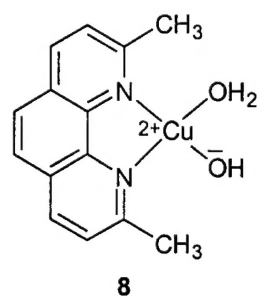
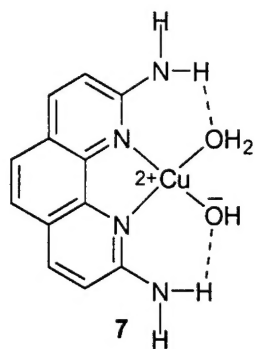
Leaving group effect. In nature there are many important phosphates with good leaving groups (ATP, soman etc) as well as those with poor leaving groups (DNA, RNA, VX etc). Thus it is of considerable interest to understand the mechanism of hydrolysis of both types of phosphates. We studied the hydrolysis of both types of phosphates bound to dinuclear cobalt complexes (1 - 4) and obtained valuable informations on the extent of bond making and bond breaking at the transition states.^{1,2}



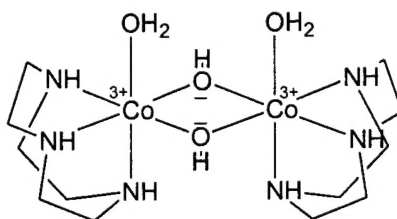
Phosphates with good leaving groups hydrolyze more rapidly in **5** than in **6**.³ In contrast, phosphates with poor leaving groups hydrolyze more rapidly in **6** than in **5**. Strongly basic leaving groups should be protonated at the transition state of the hydrolysis reaction while weakly basic leaving groups need not be protonated. In **6**, proton transfer can take place from the nucleophile to the poor leaving group (**6a** to **6b**). In **5**, proton transfer cannot take place from the nucleophile to the poor leaving group since we have shown that the nucleophile is the bridging oxide. It therefore appears that phosphates with poor leaving groups hydrolyze more rapidly in **6** than in **5** because proton transfer can take place from the nucleophile to the leaving group in **6** but not in **5**.



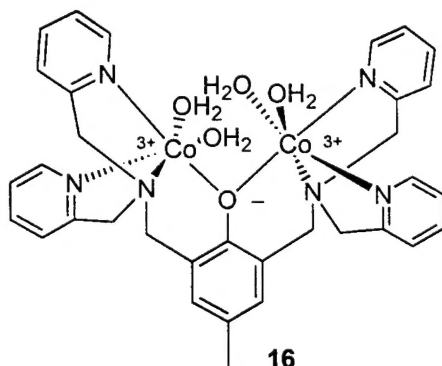
Hydrogen bonding. We have found that H-bonding can play an enormously important role for hydrolyzing phosphates with poor leaving groups. For example, **7** can be tens of thousands of times more reactive than **8** for hydrolyzing phosphates with poor leaving groups.⁴ Similarly we find that **9** is much more reactive than **10** for hydrolyzing phosphates with poor leaving groups. Currently there is considerable interest in metal complexes with internal H-bonds (**11** – **13**).⁵⁻⁷ Many hydrolytic metalloenzymes have been shown to contain metal-bound ligands that are hydrogen bonded (**14**).⁸ Compounds **7** and **9** represent the first model compounds that provide increased reactivity with internal H-bonding to metal coordinated ligands.



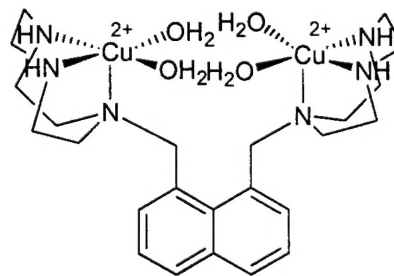
Dissecting the role of metal ions. By systematically studying the reactivity of mono and dinuclear metal complexes (**15**, **16**, **17**)⁹ it has been possible to dissect the three modes of activation that metal ions can provide for hydrolyzing phosphates. Lewis acid activation can provide about two orders of magnitude rate-acceleration while nucleophile activation and leaving group activation can provide about eight and six orders of magnitude rate-acceleration respectively.



15



16



17

References

- 1) J. Chin et al *J. Am. Chem. Soc.* (1998) p8079.
- 2) J. Chin et al *J. Am. Chem. Soc.* (1999) p3341.
- 3) J. Chin et al *J. Am. Chem. Soc.* (1998) p9943.
- 4) J. Chin et al *J. Am. Chem. Soc.* (1999) p4710.
- 5) R. H. Crabtree et al *Organometallics*. (1999) p1615.
- 6) H. Masuda et al *Angew. Chem. Int. Ed.* (1998) p798.
- 7) A. S. Borovik et al *J. Am. Chem. Soc.* (2000) p1836.
- 8) W. N. Lipscomb et al *Chem. Rev.* (1996) p2375.
- 9) J. Chin et al *Acc. Chem. Res.* (1999) p485.

List of publications

- 1) J. Chin et al *Curr. Op. Chem. Biol.* (1997) p514.
- 2) J. Chin et al *J. Am. Chem. Soc.* (1998) p8079.
- 3) J. Chin et al *J. Am. Chem. Soc.* (1998) p9943.
- 4) J. Chin et al *J. Am. Chem. Soc.* (1999) p3341.
- 5) J. Chin et al *J. Am. Chem. Soc.* (1999) p3341.
- 6) J. Chin et al *Acc. Chem. Res.* (1999) p485.

Report of invention: None

Personnel

D. Williams (PhD)
N. H. Williams (postdoctoral fellow)
W. Cheung (MSc)
B. Stranix (postdoctoral fellow)
S. J. Lee (postdoctoral fellow)
K. M. Kim (postdoctoral fellow)
J. Chin (principal investigator)

Publications from previous contracts from ARO

15 Mar '94 to 14 Mar '97

- 1) J. Chin et al *Angew. Chem. Int. Ed.* (1993) p1633.
- 2) J. Chin et al *Inorg. Chem.* (1994) p665.
- 3) J. Chin et al *J. Chem. Soc. Chem. Commun.* (1994) p1441.
- 4) J. Chin et al *Angew. Chem. Int. Ed.* (1995) p472.
- 5) J. Chin et al *Inorg. Chem.* (1995) p1094.
- 6) J. Chin et al *J. Am. Chem. Soc.* (1995) p8582.
- 7) J. Chin et al *J. Am. Chem. Soc.* (1995) p9441.
- 8) J. Chin et al *Angew. Chem. Int. Ed.* (1995) p2412.
- 9) J. Chin et al *J. Am. Chem. Soc.* (1995) p10577.
- 10) J. Chin et al *J. Chem. Soc. Chem. Commun.* (1996) p131.
- 11) J. Chin et al *J. Am. Chem. Soc.* (1996) p9982.
- 12) J. Chin et al *Inorg. Chem.* (1996) p7472.

1 July '90 to 30 June '93

- 1) J. Chin et al *J. Am. Chem. Soc.* (1993) p3618.
- 2) J. Chin et al *J. Am. Chem. Soc.* (1993) p1157.
- 3) J. Chin et al *J. Am. Chem. Soc.* (1992) p99792.
- 4) J. Chin et al *Acc. Chem. Res.* (1991) p145.
- 5) J. Chin et al *Angew. Chem. Int. Ed.* (1990) p523.
- 6) J. Chin et al *J. Am. Chem. Soc.* (1989) p4103.
- 7) J. Chin et al *J. Am. Chem. Soc.* (1989) p2724.
- 8) J. Chin et al *J. Am. Chem. Soc.* (1989) p186.

May 24, '01

Final report: July 1, '98 to June 30, '01

Artificial Metallo-Phosphoesterase

DAAG55-98-1-0398

Jik Chin

Dept of Chemistry, University of Toronto
80 St George St.
Toronto, Ontario, Canada
M5S 3H6

P-37670-CH

We investigated the role of metal ions in phosphate hydrolysis reactions with particular emphasis on understanding *the hydrolysis of phosphates with good and poor leaving groups*. We also studied the synergistic effect between *metal coordination and H-bonding in phosphate hydrolysis*. Finally, we have been able to *dissect the role of metal ions in hydrolyzing phosphates*.

Metal, phosphate, hydrogen bond, hydrolysis